signals detected after affinity capture. Once the number of such panning peptides resulting from the protease digestion of one target protein is known, that number can be easily translated into the amount of the target protein in the sample. Amino acids other than lysine can also be targeted for use with this method. For example, proteins with limited number of natural or added cysteine may be selected or constructed to be labeled, via a reduced thiol with maleimide-coupled dye such as maleimide-coupled Alexa 488 (commercially available from Molecular Probes of Eugene, Oregon).

[0100] Indirect labeling of an antigen analyte may be achieved by using a second antibody or antibody fragment that has been labeled for subsequent detection (e.g., with radioactive atoms, fluorescent molecules) in a sandwiched fashion. In a preferred embodiment, an antigen that binds to a microarray of antibodies is detected through a second fluorescently labeled antibody to the antigen, obviating the need for labeling the antigen. In a further preferred embodiment, the second antibody is a labeled phage particle that displays an antibody fragment. Standard phage display technology using phages such as M13 may be used to produce phage antibodies including antibody fragments such as scFv. This allows relatively easy and fast production of reagents for sandwich detection from phage display antibody libraries. To ensure that the phage antibodies recognize an epitope different from the one that the immobilized capture antibody recognizes on the antigen, selection from phage display libraries may be carried out in the following way: (1) tubes are coated with the same antibody that is immobilized in microarray for capture purpose, (2) the tube is blocked and the antigen is added and captured by the coated antibody, (3) after washing, phage antibody libraries may be panned in the tubes. The isolated phage antibodies (or polyclonal phage antibody) will only bind epitopes distinct from the epitope the capture antibody recognizes, and are thus ideal for the sandwich detection approach.

[0101]   F. Contact time

[0102]   Binding assays can be performed by exposing samples to the surface prepared according to methods described above. Such a surface is first exposed to a sample solution and then incubated for a period of time appropriate for each specific assay, which largely depends on the time needed for the expected binding reactions. This process can be repeated to apply multiple samples either simultaneously or sequentially. Sequential application of multiple samples generally requires washes in between.

[0103]   VI. Binding Assays

[0104]   A surface prepared according to the methods described above can be used to screen for molecules in a sample that have high affinity for the binding elements attached to the surface. Specific binding may be detected and measured in a number of different ways, depending on the way the target molecules in the sample are labeled, if at all. A common example is to use the technique of autoradiography to detect binding of molecules pre-labeled with radioactive isotopes.

[0105]   In a preferred embodiment, fluorescent dyes (CY5) were used to label proteins in a given sample before the sample was applied to a slide surface printed with microarrays of functional scFv. After incubation and washes, the

slide surface was then dried and imaged on a molecular dynamics STORM or ArrayWorx™ optical reader from Applied Precision of Seattle, Wash.

[0106]   In another preferred embodiment, secondary antibodies labeled with fluorochromes such as CY3 were used for later detection of a primary antibody participating in the binding.

[0107]   Various detection methods known in the art such as mass spectrometry, surface plasmon resonance, and optical spectroscopy, to name a few, can be used in this invention to allow detection of binding even if binding targets are not labeled at all.

[0108]   VII. Analysis of Assay Results

[0109]   A. Detecting Presence/Absence in Samples

[0110]   This invention can be used to confirm the presence or the absence, in a biological sample, of a binding partner to a molecule of interest.

[0111]   B. Determining Ratios Between Samples

[0112]   Ratios of gene and protein expression in different cell populations, such as between a normal and a diseased state, can be calculated for comparison.

[0113]   VIII. Applications/Utilities

[0114]   Because the molecules of biological significance that can be studied by this invention include, but are not limited to, those involved in signal transduction, apoptosis, dimerization, gene regulation, cell cycle and cell cycle checkpoints, and DNA damage checkpoints, the present invention has broad applications in the research of biological sciences and medicine.

[0115]   As will also be appreciated by one of ordinary skill in the art, protein arrays may also be useful in detecting interactions between the proteins and alternate classes of molecules other than biological macromolecules. For example, the arrays of the present invention may also be useful in the fields of catalysis, materials research, information storage, separation sciences, to name a few.

[0116]   A. Target Discovery

[0117]   It will be appreciated by one of ordinary skill in the art that the generation of arrays of proteins having extremely high spatial densities facilitates the detection of binding and/or activation events occurring between proteins of a defined set and biological macromolecules. Thus, the present invention provides, in one aspect, a method for identifying molecular partners and discovering binding targets for macromolecules of biological significance. The partners may be proteins that bind to particular macromolecules of interest and are capable of activating or inhibiting the biological macromolecules of interest. In general, this method involves (1) providing an array of one or more proteins, as described above, wherein the array of proteins has a density of at least 1,000 spots per cm^2 (2) contacting the array with one or more types of biological macromolecules of interest; and (3) determining the interaction between specific proteins and macromolecule partners.

[0118]   In a particularly preferred embodiment the inventive arrays are utilized to identify compounds for chemical genetic research. In classical genetics, either inactivating (e.g., deletion or "knock-out") or activating (e.g., oncogenic)